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# Performance and herbivory of the tropical topshell *Trochus histrio* under short-term temperature increase and high $CO_2$

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ABSTRACT

Within tropical environments, short-term impacts of increased seawater temperature and $pCO_2$ on algae-herbivore interactions remain poorly understood. We investigated the isolated and combined 7-day effects of increased temperature (+4 °C) and $pCO_2$ (~1000 µatm) on the trophic interaction *Ulva* sp./*Trochus histrio*, by assessing: i) topshells' survival and condition index; ii) grazer consumption rates, nutritional composition and interaction strength expressed as a dynamic index. No survival differences were observed whilst body condition varied significantly. Topshells under high $pCO_2$ displayed poor performance, concomitant with lower consumption of macroalgae. Individuals exposed to increased temperature had better physical condition, thus stimulating herbivory, which in turn was negatively correlated with carbon and nitrogen contents. The dynamic index was temperature- and $pCO_2$- interactively dependent, suggesting lower grazing pressure under single acidification. Despite some limitations inherent to a short-term exposure, this study provides new insights to accurately predict tropical species' phenotypic responses in a changing ocean.

## 1. Introduction

Climate change-related stressors (e.g. warming, acidification and hypoxia) have direct impacts on biogeography, physiological performance and behavior of marine invertebrates (Hoegh-Guldberg and Bruno, 2010; Doney et al., 2012; Kroeker et al., 2013). As a result, they can alter species abundances, interaction strengths, food web topologies as well as community functioning (Alsterberg et al., 2013; Kroeker et al. 2013). Despite increasing climate change reports and body of literature towards the assessment of the ecological and socio-economic impacts derived from climate change (Hoegh-Guldberg and Bruno, 2010; Doney et al., 2012; IPPC et al., 2014), several gaps of knowledge still remain. Forecasting such impacts requires a thorough and long-term understanding of how they will affect species individually but most important the nature of species interactions (Nagelkerken & Munday, 2016), which is a trait ecologically more realistic and relevant instead studying isolated elements of a food web.

Predicting the combined effects of ocean warming and acidification is not easy, since warming can either balance the effects of acidification or exacerbate it (Kroeker et al., 2013), depending on many factors, such as type of taxa, life history characteristics, life stage, etc. Previous studies evidenced that warming is expected to strengthen top-down control through higher herbivory (O'Connor, 2009). However, under a combined scenario of warming and ocean acidification, the responses may vary greatly according to, for example, the type of organism (calcifying or not), among others. To our knowledge most of the studies focus on individual species and a growing attention has been given to predator-prey interactions, with special emphasis on behavioral changes that might occur in a rapidly changing environment (Nagelkerken & Munday, 2016). However, it is also critical to understand the combined effects of multiple climate stressors at different levels of the trophic web, particularly on primary producers' species in association with their grazers. This type of interaction still remains poorly understood and mostly restricted to temperate systems. Thus, improving knowledge on this issue will contribute for a better understanding of food web interactions and community dynamics, allowing to infer about their resilience against climate change, and ultimately the overall ecosystem processes and functioning.

Some works, mostly from temperate systems, like the one from Sampaio et al. (2017), revealed different performances of the gastropod *Gibulla umbilicalis* and the crustacean *Melita palmata* when exposed to increased temperature and acidification. Increases in temperature and $CO_2$ have benefited *G. umbilicalis,* by lowering *M. palmata* survival, and promoted an increase on the grazing pressure of the consumers on algal

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**Table 1**
Seawater physico-chemical parameters in experimental setups. Salinity and temperature were measured daily and averaged per replicate aquarium over the whole experimental period. The combination of total alkalinity (AT) and $pH_T$ (pH total scale) was used to calculate carbonate system parameters $pCO_2$ (carbon dioxide partial pressure), $C_T$ (total inorganic carbon) and ΩAr (aragonite saturation state). Values are represented as mean ± standard deviation.

| | 27 °C | | 31 °C | |
|---|---|---|---|---|
| | Ambient $pCO_2$ | Increased $pCO_2$ | Ambient $pCO_2$ | Increased $pCO_2$ |
| *Measured* | | | | |
| Temperature (°C) | 27.12 ± 0.31 | 27.18 ± 0.36 | 31.04 ± 0.38 | 31.11 ± 0.32 |
| Salinity | 35.37 ± 0.73 | 35.11 ± 0.85 | 35.32 ± 0.76 | 35.03 ± 0.81 |
| $pH_T$ | 7.98 ± 0.06 | 7.62 ± 0.03 | 8.00 ± 0.03 | 7.64 ± 0.05 |
| $A_T$ (μmol $kg^{-1}$ SW) | 1967.52 ± 48.42 | 1982.61 ± 44.90 | 1941.84 ± 50.87 | 1966.87 ± 46.18 |
| *Calculated* | | | | |
| $pCO_2$ (μatm) | 391.4 ± 21.98 | 1038.10 ± 62.57 | 360.20 ± 33.81 | 982.90 ± 60.12 |
| $C_T$ (μmol $kg^{-1}$ SW) | 1522.40 ± 31.25 | 1760.40 ± 58.22 | 1436.20 ± 71.68 | 1708.20 ± 61.82 |
| Ω Ar | 2.80 ± 0.16 | 1.41 ± 0.09 | 3.24 ± 0.14 | 1.68 ± 0.08 |

biomass (Sampaio et al. 2017). Other recently published work from the team (Cardoso et al., 2017) evidenced that co-occurring increase of temperature and $CO_2$ has promoted higher consumption rates of the macroalgae *Ascophyllum nodosum* by the gastropod *Littorina obtusata*. Along with changes in faunal communities, increased $CO_2$ levels in the ocean can modify algal nutritional quality and palatability (e.g. protein content, C:N ratio and carbohydrates) (Duarte et al., 2016; Poore et al., 2016), with likely indirect consequences for algal grazers' food preference, consumption rates and overall fitness.

The topshells of the genus *Trochus* spp. are top-shaped sea snails living in intertidal and shallow subtidal habitats in tropical Indo-Pacific coral reefs (Lee and Lynch, 1997). These gastropods feed mainly upon microalgae and diatoms attached to the rocks and/or substrate surfaces but also on macroalgae, including Chlorophyceae (Lambrinidis et al., 1997). Topshells are a very popular seafood in the Asian and Pacific regions and their shells are used as raw materials by the nacre button industry, making it among the most valuable marine snails (Ramakrishna and Sivaperuman, 2010). Despite their high abundance and socio-economic importance, much is still unknown regarding *Trochus* spp. ecology (Jolivet et al., 2015), which represents an opportunity to improve knowledge and to determine their potential as biomodels in order to evaluate climate-related changes over species trophic ecology. Regarding the studied species, *Trochus histrio*, there is a recent study investigating gender differences in the oxidative stress status under increased temperature and $CO_2$-induced seawater acidification (Grilo et al., 2018). However, this work did not explore the trophic relationship between *T. histrio* and the primary producer species, which further strengthens the ecological relevance of the present study.

Therefore, we investigated, for the first time, if a short-term increase in seawater temperature and high $CO_2$ (as single stressors and combined) could alter the *T. histrio/Ulva* sp. trophic interaction by assessing: i) topshells' survival and condition index; ii) grazer consumption rates, nutritional composition and interaction strength expressed as a dynamic index. The main questions addressed in this manuscript are: 1) will combined increase in temperature and $CO_2$ have different impacts on grazer (e.g. survival, body condition and herbivory) and macroalgae traits (e.g. nutritional composition)? And 2) how will the macroalgae-herbivore interaction be modulated by the combination of both stressors? Given the scarcity of available knowledge addressing the impact of multiple stressors on algae-herbivore interactions within aquatic ecosystems, in particular tropical ones, our work has the potential to provide a new insight and serve as a pilot study for further research on the topic.

## 2. Materials and methods

### 2.1. Organisms collection and laboratory acclimation

Topshells (*T. histrio*) and *Ulva* sp. were collected by hand by local fishermen from the Indo-West Pacific region (8°11′S 114°49′W, Bali coastline, Indonesia), in late summer of 2015. After collection, organisms were shipped, under controlled conditions to aquaculture facilities of Laboratório Marítimo da Guia (LMG, Cascais, Portugal) in cooled boxes with aerated water from the local of capture. Transportation lasted 2 days and was ensured by Tropical Marine Centre UK, a marine aquarium wholesaler recognized for its efforts on the sustainable fishing of reef organisms and promotion of animal welfare. Upon arrival to the experimental facilities of LMG, and before starting the experiment, organisms were laboratory-acclimated during 1 week under seawater conditions mimicking those at collection site:: salinity = 35 (V2 refractometer, TMC Iberia, Portugal); water temperature = 27 °C (TFX 430 Precision Thermometer, WTW GmbH, Germany) and $pCO_2$ ~400 μatm/pH = 8.0 (SG8 – SevenGo pro™ pH/Ion meter, Mettler-Toledo International Inc., Switzerland).

### 2.2. Experimental set-up

After the initial acclimation to laboratorial conditions, a full factorial design manipulating temperature, $pCO_2$ and food chain length (macroalgae only and macroalgae + grazers) was considered and set in place for experimental procedures. Topshells and the macroalgae (*Ulva* sp.) were short-term (7 d) exposed to four distinct experimental treatments (see Table 1). Future scenarios of altered temperature and $pCO_2$ were set following IPCC's Representative Concentration Pathways (RCP) scenario (IPCC, 2014) and within the range of nearshore temperatures observed in the natural habitat of the target species (Pearce & Feng, 2013). Before starting the 7 day-exposure period, the pH, as a proxy of $pCO_2$, was slowly decreased, at a rate of 0.1 units per day, until reaching the desired pH (from 8.0 to 7.6). Likewise, temperature was gradually increased at a rate of 1°C per day, until reaching the highest temperature (from 27 to 31 °C).

The duration of exposure to high temperature and $pCO_2$ (i.e. 7 days) was adjusted in order to be as realistic as possible, by i) simulating short-term events of increased seawater temperature already reported in the natural habitat of *Trochus* species (Pearce and Feng, 2013); and ii) regulating exposure to climate stressors based on the ephemeral nature of *Ulva* spp. (i.e. "green tides" formed by *Ulva* spp. usually occurred for a period shorter than 2 weeks), as demonstrated by Buapet et al. (2008), who examined the effect of dissolved nutrients on uptake rates, growth, chlorophyll and tissue nutrient concentration during the same timeframe we used.

The experiment was performed in 12 recirculating seawater

aquaculture systems (3 per treatment, 55 L each), following schematics in Grilo et al. (2018) and presented in Figure S1, attached in supplementary material. Each system included 12 drilled (to allow water circulation) cylindrical plastic containers (5.5 cm diameter x 14 cm height/220 mL; rate of water renewal in each cup of 0.5 $mL{\cdot}s^{-1}$) comprising i) 6 sub-replicates with one topshell each + one piece of algae (100–150 mg ww each) and ii) 6 sub-replicates corresponding to the autogenic control (only with pieces of algae of similar weight of those in containers with the topshells). In order to avoid escape by animals, a transparent acrylic plate covered each sub-replicate. Taking into consideration the intertidal to shallow subtidal life habit of topshells (Lee and Lynch, 1997), we ensured they were not permanently submerged during the entire experiment timeframe, leaving a 5 cm-air space (i.e. between incubation seawater surface and covering acrylic plate), in order to allow animals the possibility to emerge whenever necessary.

Natural seawater was pumped directly from the sea into a storage seawater tank (5 $m^3$ total capacity). Subsequently, natural seawater was 0.35 μm filtered (Harmsco, Florida, US) and UV-irradiated (Vecton 600, TMC Iberia, Portugal), before being supplied to experimental setups. Photoperiod was set according to prevailing natural light conditions at time of collection (~12h L/12D, light/dark cycle).

Tank illumination was provided through an artificial overhead lightning apparatus (Aquabeam 1500 Ultima NP, TMC Iberia, Portugal), consisting of 2 × Cree® XT-E Fiji Blue, 4 × Cree® XT-E Ocean White and 4 × Osram Oslon® NP Blue LED's, with a correlated color temperature of 20.000 K, total luminous flux of 1965 lumens and a 120° beam angle. Seawater temperature (TFX 430 Precision Thermometer, WTW GmbH, Germany) and salinity (V2 refractometer, TMC Iberia, Portugal) measurements were performed daily, using handheld equipment. Additionally, seawater temperature was controlled by means of an automatic seawater chilling system (Frimar, Fernando Ribeiro Lda, Portugal) and submerged heaters (200 W, Eheim GmbH & Co. KG, Germany). The pH values, as a proxy of $pCO_2$, were adjusted automatically (solenoid valves), by means of a computerized controlling system (Profilux 3.1N, GHL, Germany) connected to individual pH probes. Monitoring was performed automatically every 2 s and pH values were lowered by injection (via wood air stones) of a certified $CO_2$ gas mixture (Air Liquide, Portugal) or upregulated by aeration with $CO_2$ filtered atmospheric air (using soda lime, Sigma-Aldrich). Levels of ammonia, nitrites and nitrates were monitored daily using colorimetric tests (Salifert Profi Test, Holland), remaining below detectable levels during the entire experimental period.

Following procedures described in Grilo et al. (2018), the quantification of pH was accomplished via a Metrohm pH meter (826 pH mobile, Metrohm, Germany) connected to a glass electrode (Schott IoLine, SI analytics, ± 0.001) and calibrated with TRIS-HCl (TRIS) and 2-aminopyridine-HCl (AMP) (Mare, Belgium) seawater buffers. Seawater carbonate system speciation from total alkalinity (spectrophotometrically at 595 nm) and pH measurements (Sarazin et al., 1999) was provided in Table 1. The $pCO_2$ values were calculated using the $CO_2$SYS software (Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, USA).

### 2.3. Topshells' survival and condition index

Survival was assessed by counting the number of living *T. histrio* individuals at the end of the experiment and expressed as survival rates (%). A total of 18 individuals (6 for each replicate) was considered to assess survival rates for each experimental treatment. At the end of the experiment and immediately before shell removal, topshells from all treatments were measured by using a caliper. Two diameters (D1 and D2) were recorded following procedures described in Lemouellic and Chauvet (2008) and included in supplementary material section (Fig. S2). D1 starts at the hollow of the crown basis and it is considered the most robust measurement. D2 is the longest possible diameter (D2 > D1) and the measurement most used by fishermen (Lemouellic and Chauvet, 2008). In order to guarantee homogeneity, topshells used in the present study had similar size/mass among treatments. Diameter of all topshells ranged between 2-3 cm and had averagely 3.0 grams (wet weight).

The wet weight of topshells was recorded by using an analytical balance and the excess of water outside and inside the shell was removed by using paper towels. Estimation of condition index ($CI_{weight}$) was done at the end of the experiment, following the methodology described by Hickman and Illingworth (1980). Snails were anaesthetised in a freezer at −20 °C prior to weight measurements. Afterwards, the organisms were left to defrost slowly, at ambient temperature, during 1h, weighed (whole weight) and then cracked in order to separate soft tissues from the shell. These procedures were applied for all the organisms, across all treatments, and performed by the same person to minimize errors. The soft tissues were dried for 48 h at 50 °C to obtain a dry weight measurement. The values were used to calculate the $CI_{weight}$ by means of the following equation (Hickman and Illingworth, 1980):

$$CI = \frac{100\ x\ dry\ tissue\ weight\ (g)}{whole\ weight\ (g) - shell\ weight\ (g)}$$

### 2.4. Grazer consumption rates, macroalgae nutritional composition and interaction strength expressed as a dynamic index

Before starting the experiment, the organisms were starved for 24 h in order to eliminate the influence of previous diet and guarantee the consumption of macroalgae during experiment, according to Sampaio et al. (2017). In addition, the macroalgal pieces (i.e. with topshells and the autogenic controls with no animals to obtain estimates of the likely changes in algal mass that were not due to herbivory during the feeding assay, including algal growth) were gently dried using paper towels and subsequently weighed using an analytical balance ( ± 0.0001g precision). Likewise, at the end of experimental trials the remaining pieces of algae that were not consumed by snails and the autogenic controls were reweighed in order to determine mass changes during the experiment. Six algal pieces for each treatment were used to determine grazer consumption rates. Mass loss was adjusted for possible changes in the absence of herbivory by subtracting the loss in one of the controls lacking grazers from each replicate measure (Poore et al., 2016). Consumption rates (C) were calculated using the following formula based on Taylor and Brown (2006):

$$C = \frac{T_i\ x\ (C_f/C_i) - T_f}{(n_{bio}\ x\ t)}$$

where, $T_i$ and $T_f$ are the initial (0 days) and the final (7 days) algal biomass wet weight (ww), respectively, $C_i$ and $C_f$ are the equivalent wet weight of the control pieces, $n_{bio}$ is the wet biomass of living grazers (without the shell) in each plastic container at the end of the experiment and t is the time elapsed in the experiment (7 days).

Total carbon and nitrogen contents were measured on 6 pieces of algae of each treatment from the autogenic control containers. Algal pieces were freeze-dried, ground and combusted at 1020 °C in oxic conditions for subsequent analysis using a Delta V Advantage Isotope Ratio Mass Spectrometer coupled to an Organic Elemental Analyzer Flash 2000, Thermo Scientific (www.marinnova.pt). The nitrous oxides were reduced to $N_2$ with elementary copper at 650°C. Residual water resulting from combustion was retained in an appropriate column, in order to avoid being redirected to the mass spectrometer. After separation on a Porapak QS column, $CO_2$ and $N_2$ were detected on a TCD detector.

Grazing pressure and simultaneous macroalgal growth were analysed. In addition, to assess the strength of macroalgae-herbivore interaction, it was determined the dynamic index (Berlow et al., 1999;

O'Connor, 2009; Mertens et al., 2015), adapted to our study, according to the following formula:

$$DI = \frac{\ln(N/D)}{(n_{bio}\ x\ t)}$$

where DI is the dynamic index, N is the algal wet biomass with grazers, D is the algal wet biomass on treatments without grazers, $n_{bio}$ is the wet biomass of living grazers (without the shell) in each plastic cylinder (i.e. we chose to use biomass instead of the number of living organisms in each cylinder since we only had 1 individual per cylinder and the biomass was a more reliable measure), and t is the known period of time elapsed (i.e. 7 days). According to O'Connor (2009), this index provides a direct measure of interaction strength, i.e. the absolute value of daily per capita interaction strength, accounting for differences in algal growth rates with and without grazers. For interpretation, negative dynamic index values indicate that one species reduces the abundance of the other species, thus lower values reflect a stronger interaction strength (i.e. higher grazer pressure) (Sampaio et al., 2017). This index has been proved to be representative of survival rates and metabolic alterations associated with climate change stressors into an ecological response (O'Connor, 2009). In comparison with other methods, DI does not consider equilibrium between algae and grazers, which is advantageous for relatively short experiments (Berlow et al., 1999; Sampaio et al., 2017), as it is the case of our study.

### 2.5. Data analysis

Prior to analyses of variance, data were tested for normality and homoscedasticity using Kolmogorov-Smirnov and Levene's tests, respectively. In order to detect significant differences in *T. histrio* survival rates and body condition at the end of exposure, a two-way ANOVA was performed, using $pCO_2$ (2 levels; ambient and increased) and temperature (2 levels: 27 °C and 31 °C) as fixed factors (n = 18 for survival, n = 6 for condition index). Differences in the consumption of macroalgae and nutritional composition were also assessed through a two-way ANOVA, including temperature and $pCO_2$ as fixed factors (n = 6). Whenever the ANOVA revealed significant differences, Tukey *post-hoc* tests were applied to better scrutinize the effect of explanatory variables on each measured endpoint. A Pearson correlation analysis was applied in order to investigate potential relationships between nutritional composition of *Ulva* tissues in carbon and nitrogen and topshells' consumption rates. All statistical analyses were performed with Statistica 7 software (StatSoft, Tulsa, OK, USA).

## 3. Results

### 3.1. Topshells' survival and body condition

Short-term exposure of *T. histrio* to elevated temperature and $pCO_2$, separately and in combination, resulted in very high survival rates (Fig. 1A). At the end of exposure, the lowest survival rates were observed under increased $pCO_2$ (~90%; 2 individuals died), followed by topshells exposed to present-day conditions (~95%; 1 individual was found dead). During 1-week exposure, no mortality occurred under elevated temperature, whether alone or combined with increased $pCO_2$. No survival differences were detected between distinct temperatures and $CO_2$ treatments (Table S1 in supplementary material).

Body condition of topshells varied significantly according to different temperatures and $CO_2$ treatments, as single variables and also interactively (Fig. 1B, Table S2 in Supplementary material). Isolated high $pCO_2$ caused a significant body condition decline, which was counteracted by elevated temperature. In fact, organisms exposed to higher temperature showed better physical body condition than those exposed to present-day conditions, similar to the natural environment.

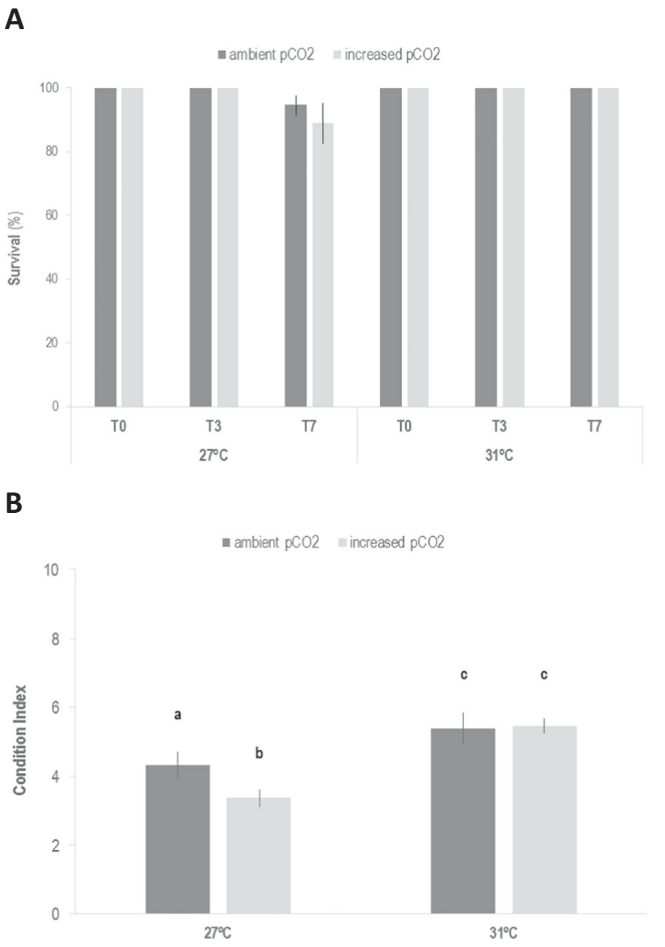


**Fig. 1.** Survival and body condition of *Trochus histrio*. A) survival rates (% ± SD; n = 18 per treatment). B) condition index (mean ± SD; n = 6 per treatment). Experimental conditions: ambient (27 °C | ambient $pCO_2$); high $pCO_2$ (27 °C | increased $pCO_2$); elevated temperature (31 °C | ambient $pCO_2$); elevated temperature + high $pCO_2$ (31 °C | increased $pCO_2$). Different letters indicate significant differences among treatments ($p < 0.05$, Tukey's test). T0, T3 and T7 correspond to the days 0, 3 and 7.

### 3.2. Grazer consumption rates, macroalgae nutritional composition and interaction strength expressed as a dynamic index

Consumption of macroalgae was significantly modelled by temperature and by the interaction between temperature and $CO_2$ (Fig. 2A and Table S3 in supplementary material). Herbivory was stimulated under elevated temperature regardless of the $pCO_2$ at which topshells were exposed to. Moreover, higher temperature *per se* was able to reverse the decline in consumption of macroalgae caused by isolated high $pCO_2$.

Nutritional quality of *Ulva* sp., expressed as changes in carbon and nitrogen contents, was interactively affected by temperature and $pCO_2$ (Fig. 2B). Multiple pairwise comparisons revealed that at present-day temperature, increased $pCO_2$ significantly enhanced the assimilation of carbon and nitrogen by the green alga (Fig. 2B; Table S4 in Supplementary material). However, with increasing temperature, this effect was annulled. Regarding the C:N ratio there were no significant differences among the treatments, although a significant interaction between temperature and $pCO_2$ (Fig. 2B; Table S4 in supplementary material) was observed. In addition, negative correlations were found between nutrient tissue contents and grazer consumption: for carbon (Pearson correlation coefficient, $r = -0.47$; $p = 0.02$) and also for nitrogen contents of algal tissues (Pearson correlation coefficient,

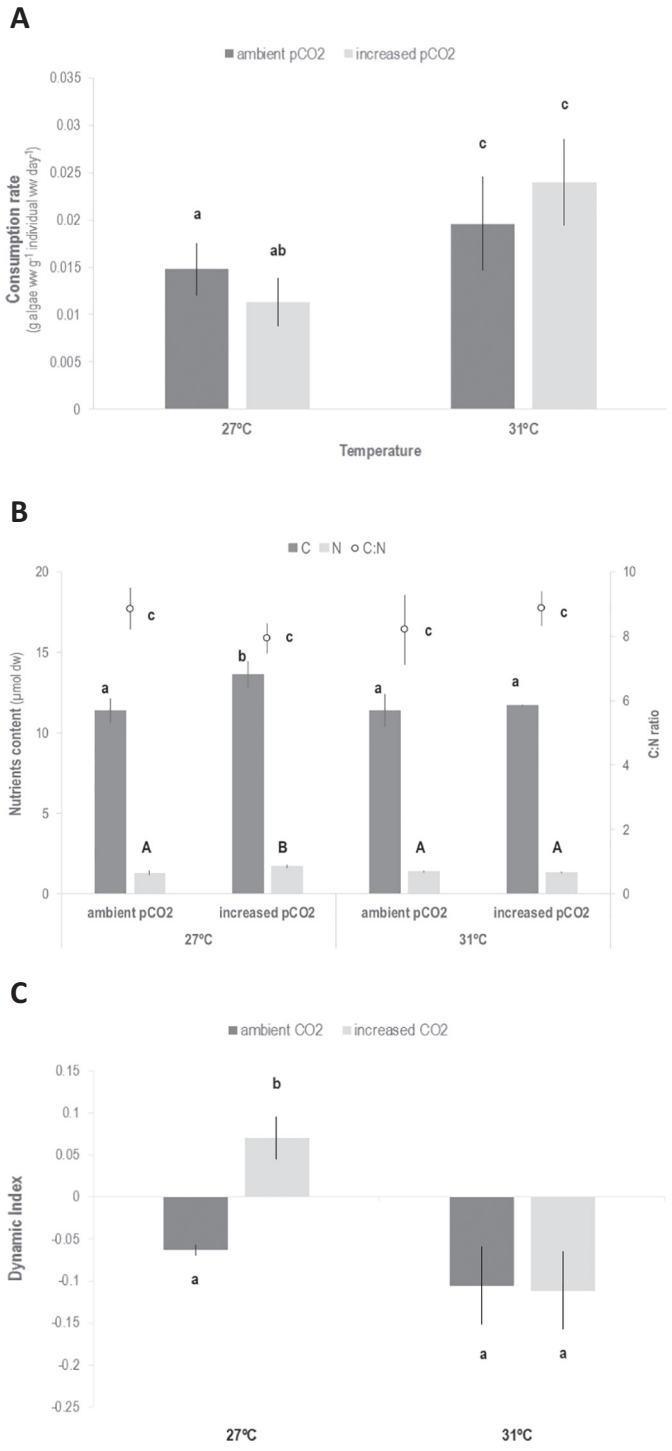


**Fig. 2.** Rates of macroalgae consumption, nutritional composition and dynamic index. A) Consumption rates (g algae ww $g^{-1}$ individual ww $day^{-1}$) of *Ulva* sp. by *Trochus histrio* (mean ± SD; n = 6 per treatment). B) Nutritional composition of *Ulva* sp. (µmol dry content in carbon, nitrogen and C:N molar ratio; mean ± SD; n = 6 per treatment). C) Dynamic Index (DI, mean ± SD) as a strength measure of the herbivore macroalgae interaction. For interpretation, negative dynamic index values indicate that one species reduces the abundance of the other species, thus lower values reflect a stronger interaction strength (i.e. high grazer pressure). Experimental conditions: ambient (27 °C | ambient $pCO_2$); high $pCO_2$ (27°C | increased $pCO_2$); elevated temperature (31 °C | ambient $pCO_2$); elevated temperature + high $pCO_2$ (31 °C | increased $pCO_2$). Different letters indicate significant differences among treatments ($p < 0.05$, Tukey's test).

r = −0.41; p = 0.04).

The dynamic index (DI) was interactively dependent on temperature and $pCO_2$, (Fig. 2C; Table S5 in Supplementary material). Under 27 °C, increased $pCO_2$ resulted in positive dynamic index values, suggesting a dominance of bottom-up control (lower grazing pressure). In opposition, for all the remaining treatments, the dynamic index assumed negative values, indicative that one species reduces the abundance of the other species, reflecting a largest top-down interaction strength and higher grazing pressure.

## 4. Discussion

The effects of $CO_2$-induced ocean acidification on survivorship of molluscs have been highlighted as quite variable and species-specific, and mostly focused on temperate bivalve species (Range et al., 2012; Hiebenthal et al., 2013; Gazeau et al., 2014). In general, ocean acidification elicited neutral (Beesley et al., 2008; Amaral et al., 2012; Range et al., 2012; Gazeau et al., 2014) or detrimental effects (Berge et al., 2006; Hiebenthal et al., 2013) on the survival of marine shelled molluscs. In the present study, although no effects were observed on survival of topshells, significant changes occurred in their body condition, manifested by poor performance under isolated high $pCO_2$ and better physical condition under higher temperature.

Within molluscs, a broad interspecific variability in body condition under ocean acidification has been reported, varying from no evident effects (Cummings et al., 2011; Clark et al., 2013; Sanders et al., 2013) to marked changes (Hiebenthal et al., 2013). Energy (obtained from feed intake) is recognized as a major component in mitigation of ocean acidification effects (Pansch et al., 2014). Declines in body condition, as a proxy for fitness-related traits of animals (Wilder et al., 2016), are usually associated with poor feed quality (Norkko and Thrush, 2006). It was shown that an abundant food supply might counteract or even overcome the negative effects of hypercapnia, as observed in adult (Melzner et al., 2011) and juvenile bivalves (Thomsen et al., 2013). *Trochus* snails were fed *ad libitum,* indicating that feed was not limited over the experiment, however a significant body condition decline was observed under isolated $CO_2$-enriched conditions, concomitant with lower grazer consumption rates. On the other hand, body condition was significantly enhanced with increasing temperature as well as the consumption of macroalgae. Our findings revealed that consumption of macroalgae was stimulated by temperature, irrespective of the $pCO_2$, in agreement with results for the temperate *L. obtusata* (Cardoso et al., 2017), but contradicting observations reported by Poore et al. (2016) for the temperate gastropod *Phasianotrochus eximius*, since no temperature effect was found on grazing activity. On the other hand, consumption of macroalgae by the gastropod *Gibbula umbilicalis* was temperature- and $pCO_2$- interactively dependent, dropping across a temperature gradient (Sampaio et al., 2017).

Herbivores are reliant on the nutritive value of macroalgae to fulfil dietary requirements and any alterations in the nutritional quality of primary producers they feed on, due to climate change, may affect biological processes, such as growth, fecundity and in turn, their fitness (Barile et al., 2004, Duarte et al., 2016). Throughout evolution, grazers have optimized their diets as a pathway to enhance survivorship either by 1) preferential consumption of macroalgae with a higher nutritional value (Barile et al. 2004; Duarte et al., 2016); 2) increasing the consumption of less nutritional items (compensatory feeding) (Cruz-Rivera and Hay, 2001) and/or 3) increasing absorption efficiency (Simpson and Simpson 1990; Duarte et al., 2016). Topshells exposed to elevated temperature consumed between 30 to 60% more algae than organisms exposed to ambient temperature, probably to compensate for a decline of the alga nutritional value and to satisfy metabolic requirements. According to the metabolic theory there is a general and predictable mechanistic link between individual-environment interactions and larger scale ecological patterns (O'Connor, 2009). A simple extrapolation of this theory considers that, as temperature increases, the rate of

basic metabolic processes will also increase (Brown et al., 2004), so it is expected that consumption rates should increase as a consequence of the metabolic demands (Sanford, 2002). In conflict with our results and the metabolic theory, Russell et al. (2013) argued that the combination of elevated temperature and high $pCO_2$ produced a decline in the amount of primary production consumed by grazers, at least in a short-term scale. Nevertheless, a longer exposure altered this effect, suggesting that the structure of future ecosystems may not be predictable using short-term experiments.

The role of $CO_2$ in photosynthesis, nutrient metabolism and cell processes of terrestrial and marine plants/macroalgae is particularly relevant. As a result of increased $CO_2$ levels in the ocean, alterations in the nutritional quality of macroalgae (e.g. C:N ratio and protein content) have been reported. Experiments conducted on *Ulva rigida* (Gordillo et al., 2001) and *Ulva pertusa* (Kang and Kim, 2016) under a $CO_2$-enriched environment revealed no alterations in soluble proteins, internal carbon, nitrogen and phosphorous contents. Reduced C:N ratios were reported for *Fucus vesiculosus* after exposure to high $pCO_2$ levels, with no consequences for nutritional quality or feeding rates by the isopod *Idotea balthica* (Gutow et al., 2014). In contradiction to the previous studies we found a significant increase in elemental carbon and nitrogen *Ulva*'s tissue contents under high $pCO_2$, which was negatively correlated with grazer consumption and that in turn was associated with poor topshells' body condition. As reported by Celis-Plá et al. (2015) ocean acidification may benefit algae that are able to capitalize on increased carbon availability for photosynthesis and it may also stimulate nitrogen assimilation depending on light and nutrient conditions.

Previous studies (Alsterberg et al., 2013; O'Connor, 2009) demonstrated that the interaction strength macroalgae-herbivore (i.e. grazing pressure) may be stimulated with increasing temperature and $pCO_2$, leading frequently to shifts in communities' dominance. However, communities' responses are complex and not well understood based on single effects, which was clearly evident through dynamic index profiles (Sampaio et al., 2017). Grazing pressure boosted along with temperature increase, as well as survival and body condition, which significantly enhanced macroalgae-herbivore interaction, resulting in a top-down dominated community. On the other hand, increased $pCO_2$, as single stressor, weakened community response by deaccelerating the interaction strength (e.g. less consumption of macroalgae and reduced body performance), leading to a bottom-up controlled community.

Summing up, coastal areas are predicted to display large and uncertain regional and local temperature and $pCO_2$ fluctuations (IPCC, 2014). In the light of these changes topshells were resilient, in a short-term, to a relatively large increment in temperature and $pCO_2$ (at least up to 4 °C and 700 µatm above their natural environment). Likewise, the tropical gastropod *Littoraria scabra* was described to be very tolerant to global warming, being able to survive up to 6 °C higher its natural environment by selecting a thermally favorable substrate over short temporal scales (Chapperon and Seuront, 2011). As a behavioural adaptation species might find suitable microclimates within their current distributions (Huey et al., 2012) or to track their niches as they move across space. This may sound somehow contradictory since tropical mobile invertebrates have been described as more vulnerable to such changes, since they are closer to their upper thermal limit (Chapperon and Seuront, 2011; IPCC 2014; Marshall et al., 2015). We might argue that *Trochus* spp. have developed efficient biochemical mechanisms in response to climate change (Grilo et al., 2018) or have adjusted either through alterations in their genetic composition (which was not possible to accomplish in this study) or by phenotypic plasticity (Goldberg et al., 2007). Either of these outcomes may lead to changes in the timing of events (phenology), morphological variation (e.g. color patterns, body shape and size), behaviour or physiological alterations, allowing the species to better cope with the impacts of climate change (Fuller et al., 2010). Nevertheless, our results are inadequate and narrow by themselves to infer about the long-term consequences of climate change on *Trochus* feeding ecology, trophic interactions, and ultimately for the whole ecosystem, as this study only took into consideration a short-term scale, which may not be sufficient to accurately predict species' phenotypic responses to future environmental changes. Quantifying the mechanisms underlying the altered interaction between *T. histrio* and *Ulva* spp. requires further analysis of metabolism, nutritional composition and concentration of chemical defenses across a range of temperatures and $pCO_2$ values in a long-term approach. A longer exposure of topshells to ocean warming and acidification would likely provide new insights about their resilience in the future.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2018.11.011.

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